Reference: *Biol. Bull.* **196:** 245–256. (June 1999)

Development, Temperature Tolerance, and Settlement Preference of Embryos and Larvae of the Articulate Brachiopod *Laqueus californianus*

J. TIMOTHY PENNINGTON*, MARIO N. TAMBURRI, AND JAMES P. BARRY

Monterey Bay Aquarium Research Institute, 7700 Sandholdt Road, Moss Landing, California 95039

Abstract. Populations of the articulate brachiopod *Laqueus californianus* occur in dense single-species aggregations near the continental shelf/slope break (100-200 m) in Monterey Bay, California. The development of embryos and larvae of *L. californianus* has been examined by scanning electron microscopy. Fertilizable eggs are 130-140 μm in diameter, and sperm are unmodified. Cleavage is holoblastic and radial. At 10°C an up-swimming blastula develops by 18-h, and gastrulation occurs within 24-38 h. The embryo elongates on a new larval axis and the blastopore closes by 72 h. A trilobed articulate brachiopod larva forms by day 3-4, and a metamorphically competent larva with attachment disk is attained in 7 days. Competent larvae swim downwards.

Effects of temperature on larval survival and development rate have also been examined. Larvae die within 1 day at 25°C. At 20°C, development appears normal but results in spontaneous abnormal settlement of larvae 5-6 days old. At 15°, 10°, and 5°C, most larvae achieve competence in 5, 7, and 9 days, respectively. Many larvae survive for 71 days at 10° and 15°C.

Patterns of larval settlement vary among substrates, but larvae show strong preference for shells of living conspecific adults. Settlement and metamorphosis can occur within 24 h upon exposure of larvae to substrate.

Introduction

The Brachiopoda compose a major part of the fossil record (~30,000 described species), yet the biology of extant brachiopods (~280 species) is understood poorly relative to that of other macrofaunal invertebrates (Rudwick, 1970; James *et al.*, 1992). This lack of knowledge exists in part because brachiopods often inhabit cryptic or inaccessible habitats and are rarely conspicuous members of communities that attract the attention of zoologists and ecologists. The dense communities of epifaunal brachiopods that dominated level-bottom, shallow-water habitats prior to the Permo-Triassic extinction are largely absent from recent seas (reviewed by Thayer, 1986; Rudwick, 1970), and numerous attempts have been made to explain this shift in abundance and diversity (Stanley, 1977; Vermeij, 1977; Gould and Calloway, 1980; Gilmour, 1981; Thayer, 1981, 1985, 1986; Valentine and Jablonski, 1983a; Law and Thayer, 1991; Rhodes and Thayer, 1991; Thayer and Allmon, 1991; Rhodes and Thompson, 1993). The present-day Brachiopoda are typically regarded as a relic phylum, with extant species living in relic or marginal habitats (James *et al.*, 1992; Rhodes and Thompson, 1993).

A striking exception to this pattern occurs in Monterey Bay, California, where populations of the articulate brachiopod *Laqueus californianus* (Koch 1848, Terebratellacea) are found as dense epifaunal 'beds' at the outer margin of the continental shelf (100-200 m; Fig. 1A-B). These aggregations occur near rock outcrops associated with the San Gregorio fault zone, but the brachiopods are also abundant on nearby mud bottoms where individuals are attached to both living and dead shells of conspecifics (Fig. 1C). Research on *L. californianus* is limited, and little information is available concerning its reproductive biology.

This paper presents (1) a description of *L. californianus* embryos and larvae as examined by scanning electron microscopy (SEM), (2) data from assays of the effects of temperature on larval survival and development rate, and (3) results of experiments on substrate preference during larval settlement. The work constitutes part of an ongoing investigation of the biology and community ecology of the *L.*

Received 29 October 1998; accepted 19 February 1999.
* E-mail: peti@mbari.org

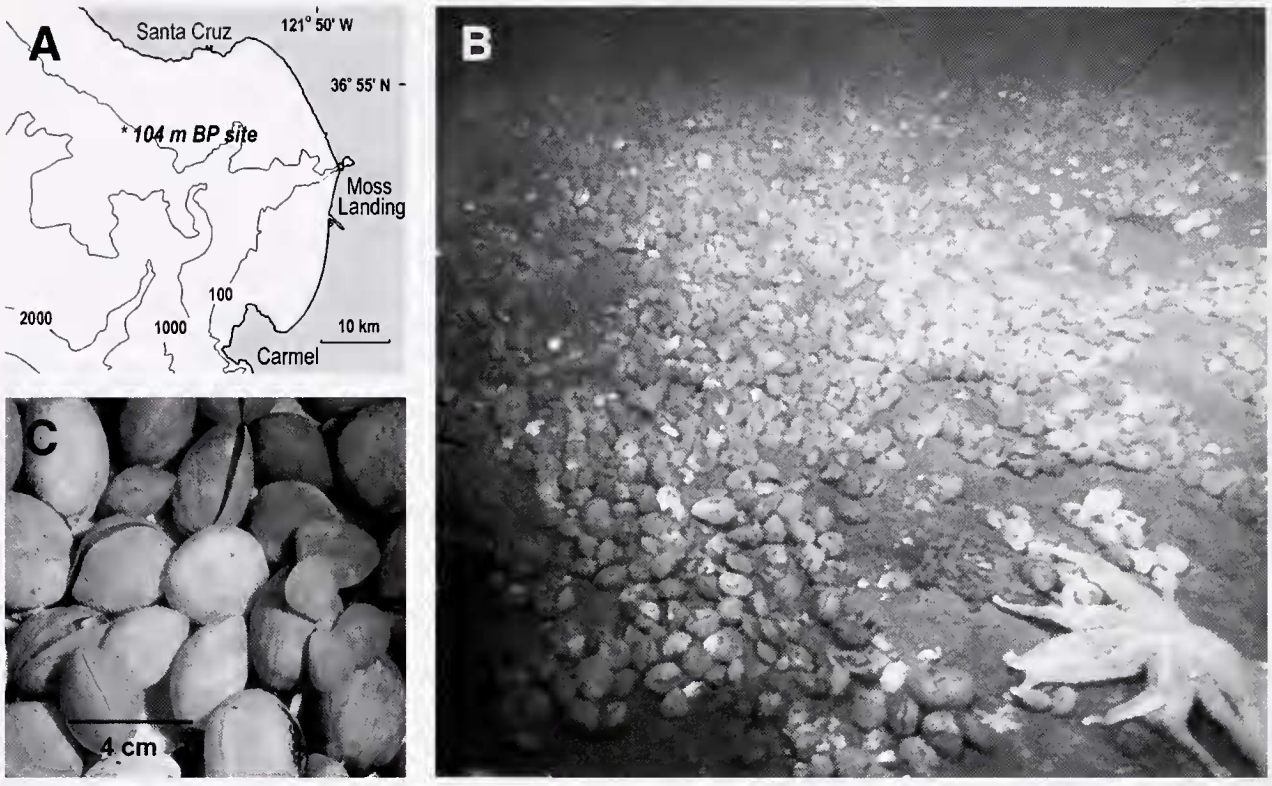

Figure 1. Collection site and habitat of *Laqueus californianus*. (A) Map of Monterey Bay, Monterey Submarine Canyon, and continental shelf and slope in central California. Contours in meters; the shelf/slope break is ~120 m deep. Brachiopods were collected from beds at 104 m in northern Monterey Bay ('104 m BP site'). (B) Submarine view of the 104 m BP site. All brachiopods are *L. californianus;* the asteroid is *Rathbunaster californicus* and is ~35 cm in diameter. Brachiopods occur on rock or nearby as beds extending over soft substrate; individual aggregations range from <1/m^2 to ~100/m^2. (C) Detail of an aggregation similar to that in B. Small brachiopods are commonly attached to larger individuals.

californianus beds in Monterey Bay—assemblages that are reminiscent of the fossil brachiopod 'reefs' described in the paleontological literature.

Materials and Methods

Collection

Adult *Laqueus californianus* were collected at a depth of 104 m by the R/V *Point Lobos* and the remotely operated vehicle (ROV) *Ventana* at the head of Cabrillo Canyon at the continental shelf/slope break in northern Monterey Bay, California (Fig. 1A). Collections were obtained with a suction sampler because most animals were attached to living or dead brachiopod shells (Fig. 1B) and were readily dislodged from the seafloor. Upon recovery of the ROV, brachiopods were placed in coolers and transported to the Monterey Bay Aquarium Research Institute (MBARI) at Moss Landing, California (Fig. 1A), and placed in a recirculating seawater system at 10°C. Adults are hardy and have been held unfed for many months prior to use in embryological work.

Gametes and rearing

Culture methods were adapted from those in Reed (1987) as developed by Long (1964) for other articulate brachiopods. All embryological and larval work was performed in filtered (5 μm) seawater obtained from surface waters in mid-Monterey Bay; embryos and larvae did not survive in recirculated seawater.

Oocytes were obtained by pressing and washing dissected ovaries through 0.5-mm nylon mesh. Oocyte suspensions were washed several times in seawater and allowed to stand overnight to undergo germinal vesicle breakdown and to shed follicle cells prior to fertilization. Sperm were stripped from testes as above and induced to swim by addition of 0.5 *M* Trisma base buffer solution to the sperm suspension (20% Trisma base:sperm suspension; pH 9; 5-30 min). Once sperm were verified motile under the microscope, oocytes were fertilized by adding ~1% by volume of faintly milky sperm suspension. Excess sperm was washed from cultures after 30 min.

Embryos and larvae were cultured in unstirred glass

beakers or gallon jars at densities of 1-10 larvae/ml. Seawater was changed daily for the first 3 days and every other day thereafter. After one day, healthy up-swimming embryos were decanted from unfertilized eggs and poorly swimming embryos at the bottom of culture vessels.

Microscopy

Aliquots of gametes, embryos, larvae, and juveniles were examined with an ISI WB-6 scanning electron microscope at 10 Kv. The samples were prepared using Karnovsky's fixative (Gold, 1976), postfixation in 1% seawater-buffered osmium, dehydration though an acetone series, critical point drying, and sputter coating with gold/palladium.

Temperature effects

Two experiments were conducted to determine survivorship and development rate as a function of temperature. Survival of larvae as a function of temperature was evaluated in assays exposing 2-day-old gastrulae to five temperatures. Capped vials containing embryos were placed in water baths at 5°, 10°, 15°, 20°, and 25°C, ±0.5°C (three replicate vials for each temperature, each vial containing 10 gastrulae and 10 ml of filtered seawater). Surviving larvae were counted daily. To minimize handling errors, culture water was not changed. The effect of temperature on development rate of larvae was assessed by rearing several hundred 2-day-old gastrulae in beakers held at the above temperatures. These larvae were scored daily for developmental stage, behavior, and general health, but were not counted. Culture water in these beakers was changed every other day.

Settlement substrate preference

Two experiments were performed to evaluate the settlement preferences of competent larvae. One experiment provided eight settlement substrates to larvae: (1) carbonate rock, (2) frosted microscope slide, (3) unidentified sedimentary rock collected from the Monterey Submarine Canyon, (4) clam shell (*Tresus nuttallii*), (5) dead, air-dried (>1 month) *L. californianus* shell, (6) dead, air-dried *L. californianus* pedicle, (7) dead, air-dried *L. californianus* shell subsequently "conditioned" in seawater for several days prior to the experiment, and (8) small (~1 cm length) live *L. californianus*. Shells in treatment (7) presumably developed a living microflora during conditioning. In this experiment, 150 competent larvae (7 days old; 10°C culture) were pipetted into each of forty 20-ml polyethylene wells. Potential settlement substrates were added to each well (~1 cm^2 exposed surface area each; five replicate wells/substrate type), and the wells placed on a shaker table (25 rpm) in a dark 10°C cold room. Larvae attached to the substrates were counted after 3 days.

A second experiment was conducted to replicate portions of the above experiment and to assess the effect of rugosity (grooves; *cf.* Wisely, 1969) and surface-bound conspecific chemical cues in larval substrate preference. Treatments in this experiment included conditioned substrates (as described above) and substrates "painted" with brachiopod extract. Painted substrates were coated five times with a solvent extract of whole *L. californianus* (five adult *L. californianus* extracted in 300 ml 100% acetone for 1 month) and allowed to air-dry > 1 week before use. Substrate treatments were (1) no substrate added, (2) painted rugose cockle shell (*Clinocardium nuttallii*), (3) unpainted rugose cockle shell, (4) painted smooth clam shell (*Tresus nuttallii*), (5) unpainted smooth clam shell, (6) painted frosted microscope slide, (7) unpainted frosted microscope slide, (8) painted smooth microscope slide, (9) unpainted smooth microscope slide, (10) conditioned smooth microscope slide, (11) conditioned rugose cockle shell, (12) dead air-dried bryozoan test (*Membranipora membranacea*), (13) dead air-dried *L. californianus* pedicle, and (14) living *L. californianus*. Thirty competent larvae (7 day-old; 10°C culture) were counted into each of forty-two 10-ml polystyrene wells. Substrates were added (three wells for each substrate type), and the wells were held in the coldroom for 24 h, after which any settled larvae were counted.

Counts in each experiment were square-root transformed to satisfy assumptions of normality and equal variance, and subsequently analyzed by ANOVA and Bonferroni *t*-tests (SigmaStat). Data from treatments in which no larvae settled were lumped into one group; because assumptions of normality and variance could not be satisfied, these data were analyzed by nonparametric ANOVA and Dunn's test (SigmaStat).

Results

Obtaining gametes

Gonads were almost always present in animals >20-25 mm in length (maximum size is ~45 mm long). Sex was not distinguishable externally, but was noted for 67 dissected adults. Of these, 44% were female, which is not significantly different than 50% (*Z*-test, $P = 0.4$). No hermaphroditism or brooding was observed. Season of reproduction remains uncertain since collections have been sporadic and few ($n = 5$). Both ovaries and testes typically appear plump following collection. Testes almost always contain at least some mature spermatozoa, and ovaries some full-size oocytes. However, oocytes from none of the field collections were immediately fertilizable, but matured when held several months in the laboratory. The most successful larval cultures were obtained in spring.

Fertilizable oocytes were 130-140 μm in diameter and surrounded by follicle cells (Fig. 2A). In such oocytes the

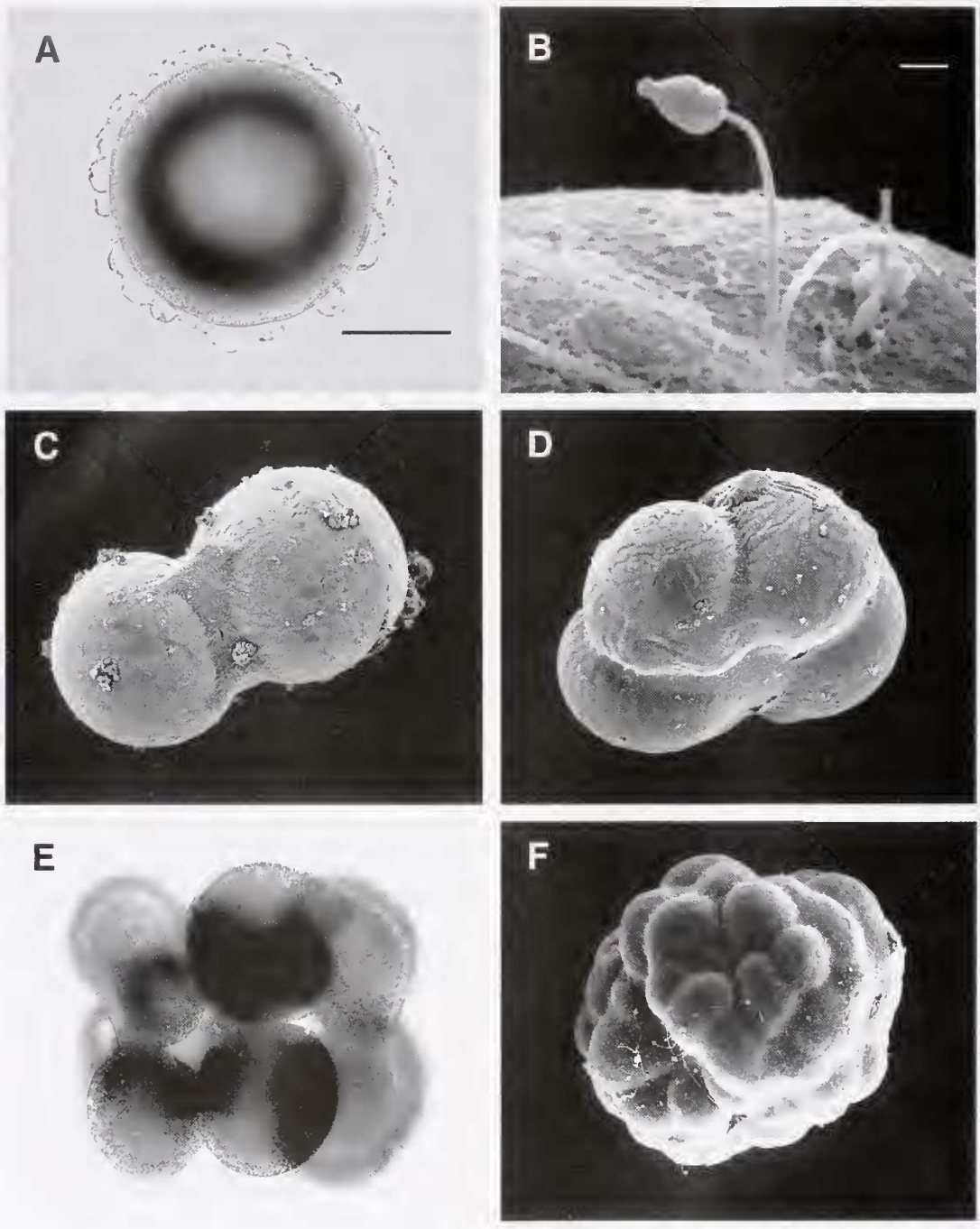

Figure 2. Early development of *Laqueus californianus*. (A) Freshly stripped oocyte. Light area in center is the germinal vesicle (nucleus) obscured by yolk; follicle cells at the periphery of oocyte slough off prior to fertilization. Scale bar, 50 μm. (B) Spermatozoan on embryo surface. Scale bar, 1 μm. (C) Two-cell embryo, with prominent extracellular membrane and a few follicle cells. Polar bodies not apparent. Scale as in A. (D) Four-cell stage. Scale as in A. (E) Eight-cell stage, showing radial arrangement of blastomeres. Small blastomere in upper left is probably abnormal. Scale as in A. (F) Late cleavage, with overlying blastomeres apparently pressed by egg membrane into furrows between cells beneath. Scale as in A.

germinal vesicle (visible as a translucent area in the salmon-colored egg cytoplasm; Fig. 2A; see Stricker and Folsom, 1997) disappeared overnight, as did follicle cells. Unfertilizable immature oocytes were more firmly attached to the ovary and when stripped were usually recognizable by a large surface dimple that probably marks the site of attachment to the ovarian wall.

Sperm heads and midpiece together are 2 μm long with

head and midpiece visible (Fig. 2B); their tails are 40-45 μm long.

Development

Embryos. A developmental schedule for *L. californianus* at 10°C is presented in Table I. Cleavage is holoblastic, equal, and radial (Fig. 2C-E). First cleavage occurs at 3 h post-fertilization, and subsequent cleavages follow at 1-h intervals until cell counts become problematic after the fourth cleavage. Polar bodies were looked for but not recognized (*cf.* Freeman, 1993a). A thick egg membrane (~2 μm; visible in Fig. 2A, C, E) appears to press blastomeres into the furrows of underlying cells in later cleavage stages (Fig. 2F).

A hollow blastula forms by 12 h (Fig. 3A) and becomes ciliated by 18 h post-fertilization. Over the next several hours, embryos swim to the surface of cultures, spiraling with a clockwise rotation (anterior view). Blastulae occasionally stop swimming when they contact the water surface. Over 20-34 h, embryos gastrulate. The blastopore opens widely at the posterior, as defined by direction of swimming (Fig. 3B-C). By 48 h, embryos flatten and elongate ("wedge" embryos; Fig. 3D-E) and the swimming axis shifts such that, relative to swimming direction (arrows in Fig. 3B, D-F), the blastopore becomes ventrolateral. At this time an apical tuft of cilia appears (Fig. 3D, but not visible in Fig. 3E-F) on the thicker and new anterior end of the wedge embryo. During this time the blastopore also elongates and begins to fill with cells (Fig. 3D) from the posterior forwards. By 72 h the blastopore is closed.

Larvae. From 80-96 h (day 3-4) the swimming embryos differentiate into larvae 150 μm long, with apical, mantle, and pedicle lobes (Figs. 3F, 4A). The apical tuft is prominent (Fig. 4A), and dorsal and ventrolateral pairs of setal bundles develop from the posterior margin of the mantle lobe (Fig. 4C). The mantle lobe is ciliated sparsely, but ciliation of the apical and pedicle lobes remains uniform. From 96-128 h (day 4-5; Fig. 4B) the setae lengthen, ciliation is lost from the pedicle lobe, and cilia at the margin of the apical lobe form a well-defined locomotory band. Viewed from anterior, swimming larvae rotate slowly clockwise while metachronal waves pass counterclockwise through cilia of the locomotory band. As viewed through a dissecting microscope in culture bowls, larvae swim ~1 mm/s. They remain near the surface, and if disturbed can stop swimming, spread their setae, and sink; they do not swim backwards. Side, top, or bottom illumination produces no obvious photobehavior, and no pigment or eyespots are visible by light microscopy or SEM. Near the end of this period (120-128 h; day 5), the apical tuft disappears (Fig. 4B). By 145 h (day 6), the pedicle lobe begins to appear conical, and the ventral side of the mantle lobe begins to grow posteriorly to partially cover the pedicle lobe. At this time a small percentage of larvae begin to swim downwards, and many display distinctive, apparently muscular lateral flexions of the apical lobe while swimming. By 168 h (day 7; Fig. 4D-E), the ventral margin of the mantle lobe has become extended as a prominent skirt partly covering the pedicle lobe, a posteriorly directed ventral band of cilia develops on the mantle lobe, and a concave depression (the "attachment disk") forms at the terminus of the pedicle lobe (Fig. 4E). Most larvae leave the surface of cultures by 168 h and are found swimming obliquely downwards at the bottom of culture bowls. Such larvae swim, without rotating, and press the anteroventral surface of the apical lobe against the substrate. We used the presence of an attachment disk (Fig. 4E) to indicate metamorphic competence in the temperature and settlement substrate preference experiments described below.

Table I

Developmental schedule of Laqueus californianus *at 10°C.*

Hour (Day)	Event	Hour (Day)	Event
0 (0)	Insemination	48 (2)	Wedge larva; apical tuft; blastopore elongating
3 (0)	1st cleavage (2-cell)		
4 (0)	2nd cleavage (4-cell)	72 (3)	Blastopore closed
5 (0)	3rd cleavage (8-cell)	80 (3)	Lobe differentiation begins; setae appear
6 (0)	4th cleavage (16-cell)		
12 (0)	Unciliated blastula	96 (4)	Early trilobed larva
18 (0)	Ciliated blastula; upswimming begins	128 (5)	Full trilobed larva; apical tuft lost
26 (1)	Gastrulation begins	154 (6)	Pedicle lobe conical; some downswimming
34 (1)	Gastrula; large open blastopore	168 (7)	Competent larva; attachment disk complete; downswimming

Metamorphosis. Several hundred larvae successfully metamorphosed in culture, although most did not settle. Many larvae also underwent abnormal or incomplete metamorphoses. Complete metamorphosis includes cementation of the pedicle lobe attachment disk to the substrate, reversal of the mantle lobe anteriorly (Fig. 5A) so that it encloses the apical lobe (Fig. 5B), and growth of adult shell valves on the now exterior surfaces of the mantle lobe (Fig. 5B). Metamorphosis of individual larvae has not been directly followed but occurs within 24 h of addition of appropriate substrate. Larval setae are not shed immediately. Juveniles that metamorphosed normally have survived to 115 days in culture, reaching a shell diameter of ~400 μm before death.

Temperature

In an assay of survival over a range of temperature (results not figured), no embryos or larvae survived 1 day at 25°C; about 15% survived to day 7 at 20° and 15°C, when

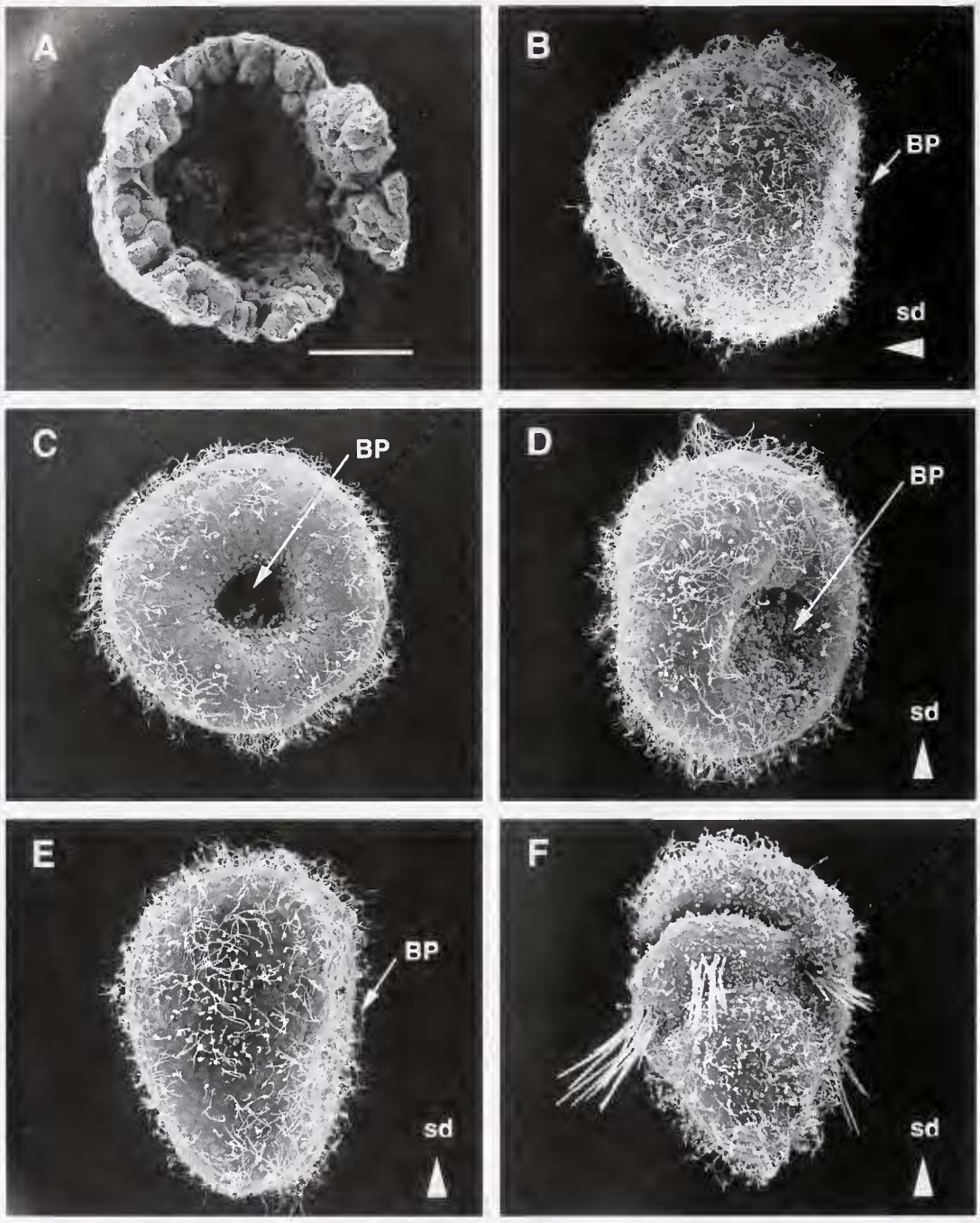

Figure 3. Late embryos and early larva of *Laqueus californianus*. (A) Broken 18-h-old blastula. Blastomeres are rounded, not yet ciliated, and ~1 layer thick beneath the egg membrane. Blastocoel is spacious and hollow. Scale bar, 50 μm; A-F to same scale. (B-C) Lateral and posterior views of 34-h-old gastrulae. Embryos are ciliated and swim to the surface of cultures with blastopore ('BP') trailing (arrows 'sd' indicate swimming direction). Blastopore open to archenteron. (D-E) Oblique ventral and lateral views of 48-h-old 'wedge' embryos. Blastopore fills with cells and closes by the first larval stage. Swimming direction changes (arrows 'sd') such that the blastopore becomes ventrolateral. An apical tuft develops (visible in D but not E-F) at the new anterior of the embryo. (F) Dorsolateral view of 80-h-old (3-day) early larva, with apical (top), mantle (middle), and pedicle (bottom) lobes differentiating. Pair of short dorsal setal bundles in foreground, and longer ventrolateral bundles to left and behind larva. Mantle lobe unciliated.

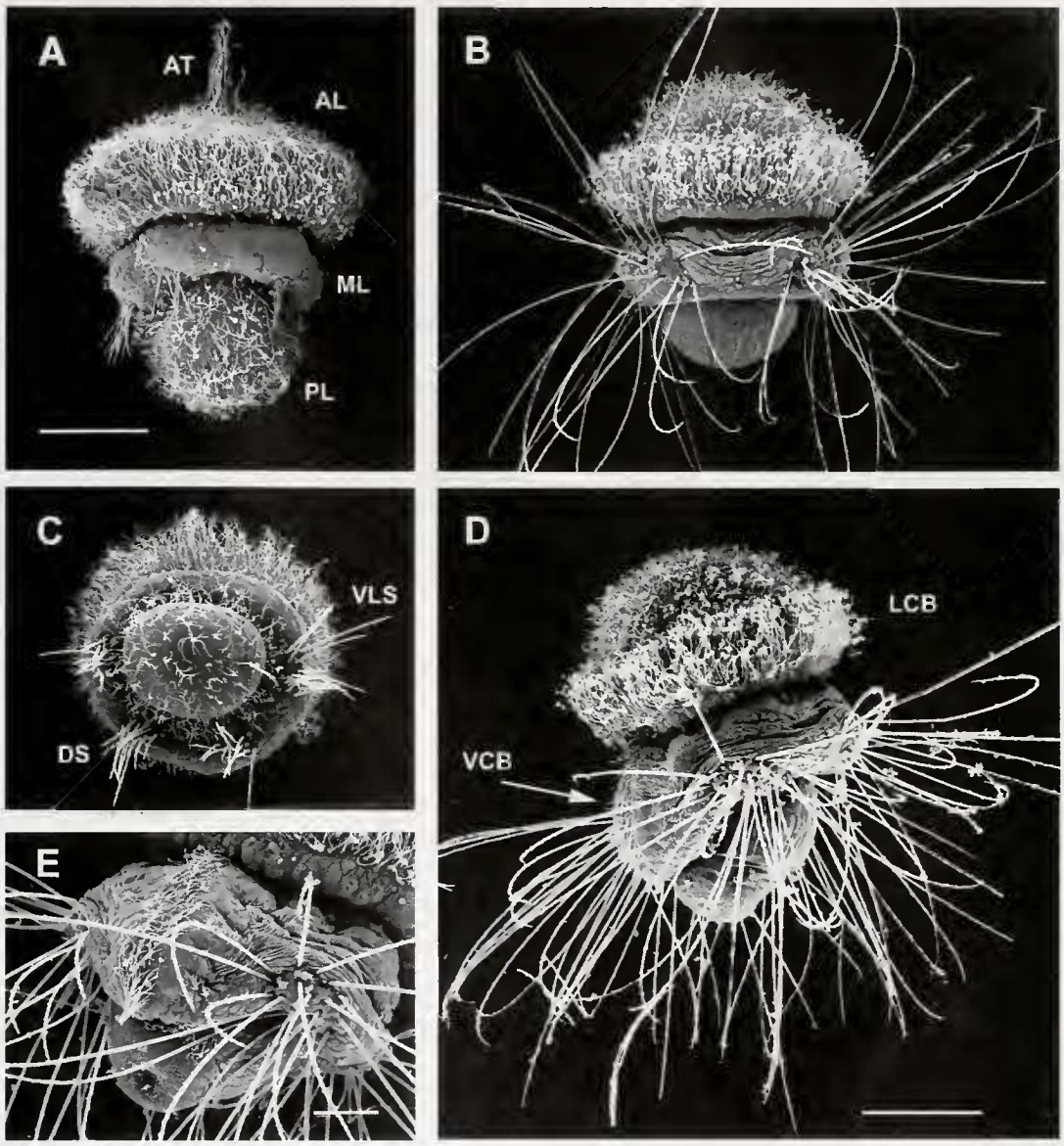

Figure 4. Larvae of *Laqueus californianus*. (A) Dorsal view of 96-h-old early larva. Apical lobe ('AL') uniformly ciliated except for prominent apical tuft ('AT'), mantle lobe ('ML') unciliated with short dorsal setal bundles and the left ventrolateral bundle visible, and pedicle lobe ('PL') rounded and ciliated. Scale bar, 50 μm. (B) Dorsal view of 128-h-old larva. Band of locomotory cilia differentiating on margins of apical lobe; the balls at tips of many cilia are probably fixation artifacts. Apical tuft cilia are present at this stage but not preserved on this specimen. Setae of mantle lobe erected, as when swimming larva is disturbed. Pedicle lobe has lost its cilia but is still rounded. Scale as in A. (C) Posterior view of 4-day-old early larva as in A. Pedicle lobe rounded and sparsely ciliated, ventrolateral ('VLS') and dorsal ('DS') setal bundles pairs on mantle lobe, and cilia on apical lobe. Scale as in A. (D) Lateral view of 168-h-old metamorphically competent larva. Locomotory ciliated band ('LCB') on apical lobe; the apical tuft of cilia is absent in competent larvae. Ventral ciliated band ('VCB') now present on mantle lobe. Pedicle lobe partially enclosed by mantle lobe; the attachment disk has formed on its distal tip. Scale bar, 45 μm. (E) Oblique ventral view of mantle and pedicle lobes of competent larva, showing ventral ciliated band and attachment disk. Scale bar, 20 μm.

these treatments were terminated; and about 80% of embryos or larvae held at 10° and 5°C survived to day 11 of the experiment. Most mortality occurred in the first 24-48 h, suggesting that either (a) late embryos (gastrulae and wedge embryos) were more sensitive to higher temperatures than larvae, or (b) lack of water changes produced the observed mortality at higher temperatures.

In a second assay, development rate increased with tem-

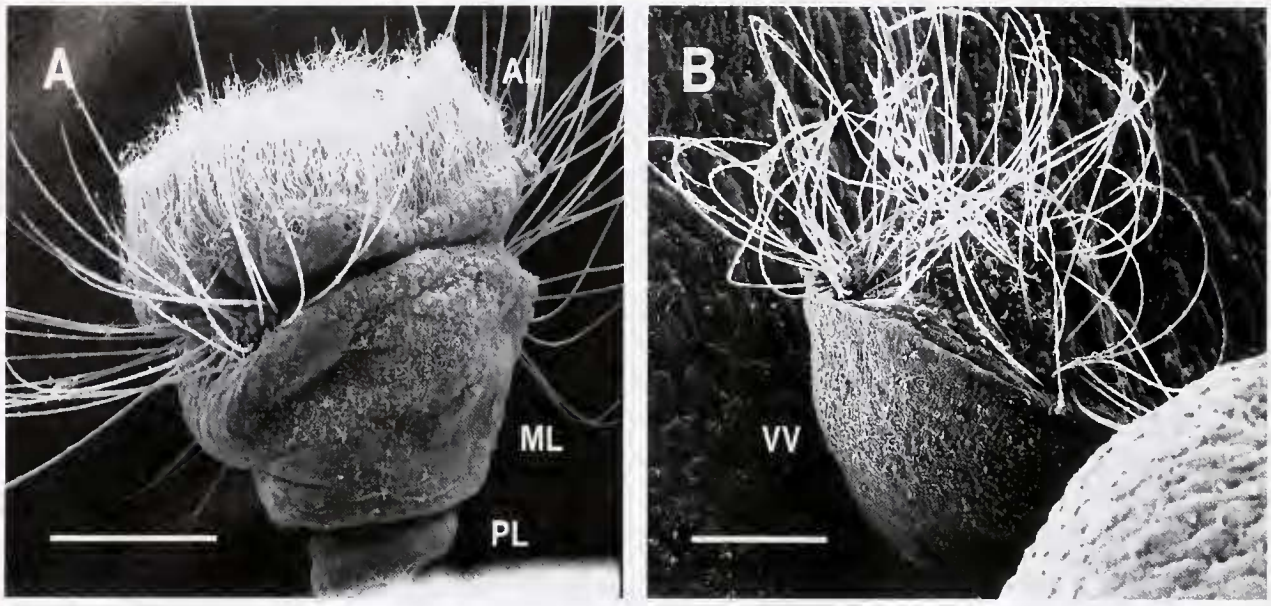

Figure 5. Metamorphosis of *Laqueus californianus*. (A) Larva undergoing mantle reversal in the first stages of metamorphosis. Mantle lobe ('ML') is beginning reversal to enclose the apical lobe ('AL'). 'PL' = pedicle lobe; scale bar, 50 μm. (B) Normal metamorphosis. Mantle reversal complete and apical lobe enclosed; shell valves secreted; 'VV' = ventral valve; scale bar, 50 μm.

perature within survival limits (Table II). Embryos held at 25°C did not develop and swam near the bottom of dishes on day 1 of the experiment. All were dead by day 2. Embryos moved from 25° to 10°C on day 1 survived for several days but did not develop. At 20°C, embryos developed into early larvae by day 1 but swam abnormally at the bottom of dishes, and by day 2 appeared competent to metamorphose. On day 3-4, most larvae cemented to the bottom of the glass culture dish but did not metamorphose (larvae 5-6 days old). Instead the mantle and pedicle lobes

Table II

Temperature effects on development rate and swimming behavior of Laqueus californianus.

Temp (°C)	Observation	Day of experiment (Age of larva)						
		1 (3)	2 (4)	3 (5)	4 (6)	5 (7)	6 (8)	7 (9)
5	Stage	Gastrula w/ AT	Early TL w/ AT	Early TL w/ AT	Full TL w/ AT	Full TL w/o AT	Full TL w/o AT	Competent
	Swim	~All up	~All up	~50% up	~50% up	Most near bottom	Most near bottom	Most near bottom
10	Stage	Gastrula w/ AT	Full TL w/ AT	Full TL w/o AT	Full TL w/ AT	Competent	Competent	Competent
	Swim	~All up	~All up	~50% up	~30% up	~20% up	~20% up	Most near bottom
15	Stage	Early TL w/ AT	Full TL w/o AT	Competent	Competent	Competent	Competent	Competent
	Swim	~All up	~50% up	~30% up	~20% up	Most near bottom	Most near bottom	Most near bottom
20	Stage	Early TL w/ AT	Competent	~70% abnormal settlement	~100% abnormal settlement	Abnormal-Terminate		
	Swim	Most near bottom	Most near bottom					
25	Stage	Gastrula w/o AT	Dead-Terminate					
	Swim	Most near bottom						

Abbreviations: AT, apical ciliary tuft; Competent, metamorphically competent larva; TL, trilobed larva.

became abnormally tall so that the apical lobe came to rest on a thin stalk ~300 μm off the bottom. This treatment was terminated on day 5. Embryos and larvae developed normally and with little mortality at 15°, 10°, and 5°C, with accelerated development at higher temperatures. Thus at 15°C, larvae became competent on day 3 of the experiment (larvae 5 days old), whereas larvae at 5°C did not become competent until day 7 (larvae 9 days old). Embryos and larvae at these temperatures swam normally at the surface of cultures, but at or near the time of attaining metamorphic competence they moved to the bottom of dishes. No larvae metamorphosed, but many survived at 10° and 15°C until day 69, when the experiment was terminated (larvae 71 days old). The higher survival of larvae in 15° and 20°C water compared to larvae in the temperature:survival assay was probably due to frequent water changes in this experiment.

Settlement substrate preference

In the first larval settlement experiment very few larvae settled on carbonate rocks, microscope slides, rocks from brachiopod habitat in the Monterey Submarine Canyon, clam shells, and dead, air-dried *L. californianus* shells (Fig. 6A). A moderate but significantly ($P < 0.05$) higher percentage settled on dead, air-dried *L. californianus* pedicles and dead, air-dried but conditioned *L. californianus* shells. In addition, a number of larvae settled on pieces of dead, air-dried bryozoan test that were inadvertently included with two of the clam-shell treatments (these settlers were scored separately and the data treated as replicates). Nevertheless, by far the largest percentage ($P < 0.05$) settled on living *L. californianus* shells. These results indicate that significantly more larvae settled on conspecifics, and further, that shells of living brachiopods were most highly preferred.

Results from the second settlement experiment confirm that larvae preferred shells of live conspecific brachiopods (Fig. 6B; $P < 0.05$), and indicate that larvae had a low preference for substrates that were conditioned or painted with brachiopod extract. These latter treatments received no settlement, and their counts were lumped in the 'other' category (Fig 6B).

Several additional experiments (data not presented) produced results consistent with those above. However, in many cases settled larvae, as scored in all settlement experiments, failed to undergo complete metamorphosis (see *Metamorphosis*, above).

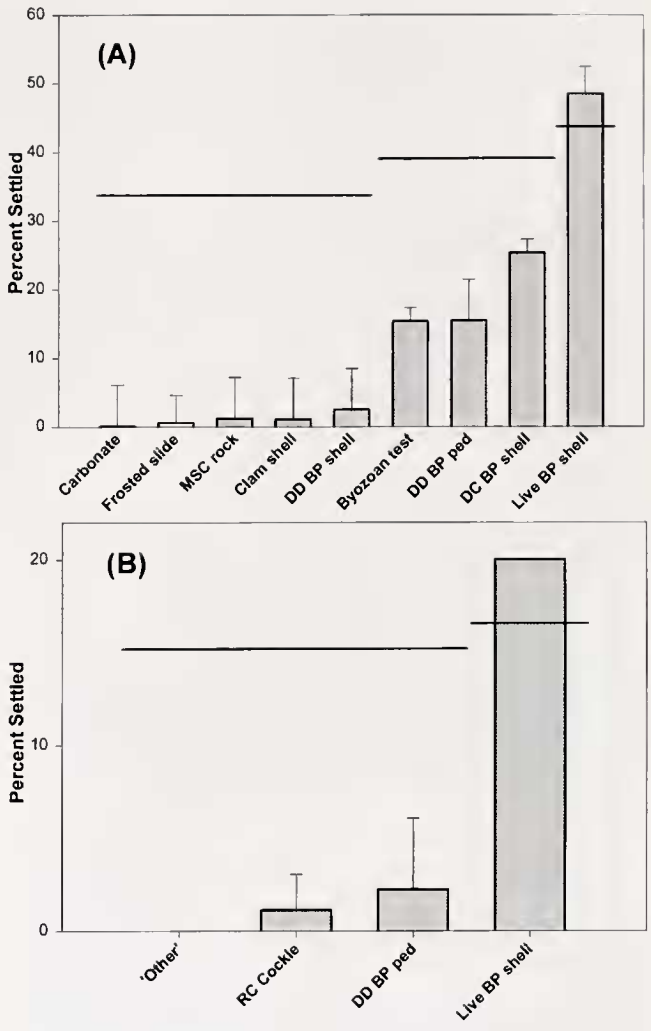

Figure 6. Mean percentage of larvae settling in substrate preference surveys. In both (A) and (B), *Laqueus californianus* larvae strongly preferred living *L. californianus* shells. Competent larvae were exposed to the various substrates and scored for settlement only; most larvae did not undergo normal metamorphosis in these experiments. Lines over histograms group nonsignificantly different treatments ($P > 0.05$); error bars are one standard deviation. Treatment substrata are detailed in Methods. Abbreviations are (A) 'MSC rock', rock collected from a brachiopod habitat in Monterey Submarine Canyon; 'DD BP shell', shell from dead, air-dried *L. californianus;* 'Bryozoan', unidentified bryozoan test; 'DD BP Ped', dead, air-dried *L. californianus* pedicles; 'DC BP shell', shell from dead, air-dried *L. californianus* conditioned in seawater; 'Live BP shell', living *L. californianus;* and (B) 'Other', 11 substratum treatments in which no larvae settled; 'RC Cockle', rugose cockle shell conditioned in seawater; 'DD BP Ped', dead, air-dried *L. californianus* pedicles; 'Live BP shell', living *L. californianus.*

Discussion

Environmental setting and adult habitat

Adult *Laqueus californianus* occur in dense epifaunal beds near the outer margin of the continental shelf in northern Monterey Bay (100-200 m; Fig. 1A-C). These beds are near carbonate rock outcrops associated with the San Gregorio fault zone (Dan Orange, University of California at Santa Cruz, pers. comm.), but often extend over or occur on near-horizontal soft sediment (Fig. 1B) where individual brachiopods are attached to either living or dead brachiopod shells (Fig. 1C) lying on mud. Other aggregations of *L.*

californianus have been observed on the continental shelf a few kilometers north of Monterey Bay, and Mattox (1955) dredged masses of adults from 60-240 m off Santa Catalina Island. Tunnicliffe and Wilson (1988) found abundant populations of *L. californianus* on vertical rock walls in British Columbia. *L. californianus* ranges from Alaska to southern California and also occurs in the Sea of Japan (Bernard, 1972; Tunnicliffe and Wilson, 1988). The species is typically found at depths <200 m (Hertlein and Grant, 1944), but is common in the intertidal zone of British Columbia (Bernard, 1972), and shell fragments have been dredged from 1570 m off the Monterey Peninsula (Dall, 1920). *L. californianus* is also common on rock walls in the Monterey Submarine Canyon to at least 800 m (Barry, unpubl), where it occurs as scattered individuals intermixed with the more ovate form *Laqueus californianus* var. *vancouveriensis*. The taxonomic status of this latter form is uncertain, but our observations of its distribution are in accord with the deeper, non-aggregated occurrence of *L. californianus* var. *vancouveriensis* reported by other authors (Hertlein and Grant, 1944; Mattox, 1955; Bernard, 1972; Tunnicliffe and Wilson, 1988). *Terebratulina crossei* also occurs in the Monterey Submarine Canyon, and the inarticulate *Glottidia albida* occurs in soft substrates on the continental shelves of Monterey Bay (Zimmer and Haderlie, 1980).

Development

Embryos and larvae. Development of *L. californianus* is similar to that of another north Pacific terebratellacean, *Terebratalia transversa* (Long, 1964; Stricker and Reed, 1985a,b,c; Long and Stricker, 1991; Freeman, 1993a,b). The eggs of *L. californianus* are smaller than those of *T. transversa* (135 *vs.* 150 μm diameter), and the sperm tails of *L. californianus* spermatozoa are longer (45 *vs.* 30 μm). Reed (1987) states that *L. californianus* eggs are 170 μm in diameter, larger than observed in this study. Cleavage and embryogenesis appear nearly identical, although we have not identified polar bodies in *L. californianus* embryos. Larvae of the two species are also similar, although *L. californianus* larvae are smaller (150 μm *vs.* 200 μm in length), do not develop pigmented eyespots, and lack vesiculated cells at the posterior margin of the apical lobe.

The developmental schedule of *L. californianus* is slower than that of *T. transversa*. At 12°-13°C, *T. transversa* reaches metamorphic competence in 4 days (Freeman, 1993a), whereas *L. californianus* takes 7 days to achieve competence at 10°C and 5 days at 15°C (Table I). We have maintained *L. californianus* larvae in culture for 71 days, which to our knowledge, is the longest recorded for articulate brachiopod larvae (also see Peck and Robinson, 1994, for a description of 45-day-old larvae of *Liothyrella uva* in the Antarctic). We do not know over what portion of this period *L. californianus* larvae remain competent to settle and metamorphose.

Development among articulate brachiopods is highly conservative (reviewed by Chuang, 1990; Long and Stricker, 1991; James *et al.*, 1992), and development in *L. californianus* is similar to that of other terebratellaceans and articulate brachiopods in general.

Metamorphosis. Metamorphosis in *L. californianus* is similar to that described for *T. transversa* and other articulates (reviewed by Long and Stricker, 1991; Chuang, 1990). However, many larvae in our cultures metamorphosed incompletely and did not proceed to develop normally. The anterior end of the apical lobe of such animals developed an ectodermal invagination (not illustrated) that is strikingly similar to stomodeal invaginations reported for other species by previous authors (Percival, 1944, 1960; Mano, 1960; Franzen, 1969). We have not followed the fate of this invagination in these abnormal individuals. Incomplete metamorphosis appears to be fairly common among articulate larvae (Percival, 1960; Freeman, 1993b) and in our cultures is probably a laboratory artifact associated with quality of eggs, culture seawater, or substratum.

Larval and recruitment biology

Depth-regulatory behavior. Changes in swimming direction associated with larval stage observed for *L. californianus* are similar to that known for a number of other articulate larvae (*Calloria inconspicua*–Percival, 1944; Doherty, 1979; Chuang, 1996; *Frenulina sanguinolenta*–Mano, 1960; *Terebratulina septentrionalis*–Noble *et al.*, 1976; *Terebratulina retusa*–James *et al.*, 1992). Such behavior has been attributed to phototaxis (*e.g.*, reviews by Long and Stricker, 1991; James *et al.*, 1992), and late larvae of many species develop putative eyespots (reviewed by Chuang, 1990). *L. californianus* larvae, however, do not have eyespots and show no obvious photobehavior in response to vertically or horizontally directed lights; their vertical swimming behaviors are probably geotactic.

Most invertebrate larvae exhibit depth-regulatory swimming (see Mileikovsky, 1973; Chia *et al.*, 1984), with young larvae typically swimming up in the water column and older larvae swimming or sinking downwards (Thorson, 1964; Young and Chia, 1988). Off central California where surface temperatures are <20°C (J.T. Pennington and F.P. Chavez, unpubl. data), *L. californianus* can develop at surface temperatures (Table II). At 1 mm/s (see Results), larvae might swim to the surface in ~28 h from brachiopod beds at 100 m. Such larvae could spend several days near the surface and could easily be dispersed dozens of kilometers by currents (see Breaker and Broenkow, 1994); the faster development at surface temperatures (Table II) should also be advantageous in terms of predation and other time-dependent sources of mortality (Rumrill, 1990). This sce-

nario contradicts the notion that articulate larvae are so short-lived that they must have very limited dispersal (Rudwick, 1970; James *et al.*, 1992), resulting in patchy meter-scale distributions of adults (especially brooding species; see Noble *et al.*, 1976; reviewed by James *et al.*, 1992). If *L. californianus* larvae can delay metamorphosis for long periods, as may be suggested by their 71-day survival in culture, widespread dispersal could occur, which could have implications for arguments concerning evolutionary rates (*cf.* Jablonski and Lutz, 1983; Valentine and Jablonski, 1983b). We plan to conduct field experiments to confirm the possibility that *L. californianus* larvae migrate to and from the surface during the course of their development, and to determine how long the larvae remain competent to settle and metamorphose.

Settlement. Late larvae of *L. californianus* swim to the bottom of cultures and engage in what may be a 'searching' behavior. In this behavior, the larvae swim, not rotating as do younger larvae, with the anteroventral surface of the apical lobe pressed against or in proximity (within micrometers) to the substrate. This anteroventral surface is at or near the leading edge of the site of blastopore closure (*e.g.*, the possible site of the adult mouth; Long and Stricker, 1991), and it is possible that the larvae are 'tasting' potential settlement sites. Similar behaviors occur among other articulate species (reviewed by Long and Stricker, 1991; James *et al.*, 1992; Chuang, 1990, 1996). Percival (1960) additionally described late larvae of *Notosaria* (= *Tegulorhyncha*) *nigricans* 'running about' the substrate by means of the ventral ciliated band.

We have not observed larvae in the act of settlement (cementation to substrate), but substrate-choice experiments (Fig. 6A-B) indicate that larvae settle preferentially on shells of living *L. californianus*. They also settle in moderate numbers on nonliving brachiopod shell and pedicle, but prefer conditioned (biofilmed) shell. Gregarious settlement is not uncommon among articulates (reviewed by Long and Stricker, 1991; James *et al.*, 1992), but has usually been inferred from distribution of juvenile recruits. Conditioned substrates, presumably colonized by microfauna, are also known to facilitate brachiopod settlement (Percival, 1960). The finding that the larvae strongly prefer living conspecific substrate, however, is new, though both Percival (1960) and Freeman (1993b) noted that live or freshly smashed conspecific shells were effective inducers of metamorphosis in *N. nigricans* and *T. transversa*, respectively. The settlement-inducing cues remain unknown—substrates treated with a brachiopod extract were apparently unattractive. Gregarious settlement would appear to be adaptive in Monterey Bay, where the brachiopod beds are largely composed of living animals cemented to each other and onto dead shell material imbedded in sediment underlying the beds (Fig. 1B-C). Larvae settling on live rather than dead shells should stand less chance of burial (but on gregariousness also see Doherty, 1979; James *et al.*, 1992). Almost certainly, preferential settlement of larvae on living conspecifics is one factor that promotes formation and maintenance of the *L. californianus* beds in Monterey Bay.

Acknowledgments

We thank D. Orange, who discovered the brachiopod beds; the crew and pilots of the R/V *Point Lobos* and the ROV *Ventana;* P. Whaling for patient collection; J. and T. Pennington for assistance with culture; K. Buck and J. Krupp for assistance with SEM; and the David and Lucile Packard Foundation for financial support.

Literature Cited

Bernard, F. R. 1972. The living Brachiopoda of British Columbia. *Syesis* **5:** 73–82.

Breaker, L. C., and W. W. Broenkow. 1994. The circulation of Monterey Bay and related processes. *Oceanogr. Mar. Biol. Annu. Rev.* **32:** 1–64.

Chia, F. S., J. Buckland-Nicks, and C. M. Young. 1984. Locomotion of marine invertebrate larvae: a review. *Can. J. Zool.* **62:** 1205–1222.

Chuang, S. H. 1990. Brachiopoda. Pp. 211–254 in *Reproductive Biology of Invertebrates. Vol. VI, Fertilisation, Development and Parental Care,* K. G. and R. G. Adiyodi, eds. John Wiley, New York.

Chuang, S. H. 1996. The embryonic, larval and early postlarval development of the terebratellid brachiopod *Calloria inconspicua* (Sowerby). *J. R. Soc. N. Z.* **26:** 119–137.

Dall, W. H. 1920. Annotated list of the recent Brachiopoda in the collection of the United States National Museum, with descriptions of thirty-three new forms. *Proc. U.S. Natl. Mus.* **57:** 261–377.

Doherty, P. J. 1979. A demographic study of a subtidal population of the New Zealand articulate brachiopod *Terebratella inconspicua*. *Mar. Biol.* **52:** 331–342.

Franzen, A. 1969. On larval development and metamorphosis in *Terebratulina* Brachiopoda. *Zool. Bidr. Upp.* **38:** 155–174.

Freeman, G. 1993a. Regional specification during embryogenesis in the articulate brachiopod *Terebratalia*. *Dev. Biol.* **160:** 196–213.

Freeman, G. 1993b. Metamorphosis in the brachiopod *Terebratalia:* evidence for a role of calcium channel function and the dissociation of shell formation from settlement. *Biol. Bull.* **184:** 15–24.

Gilmour, T. H. J. 1981. Food-collecting and waste-rejecting mechanisms in *Glottidia pyramidata* and the persistence of Lingulacean inarticulate brachiopods in the fossil record. *Can J. Zool.* **59:** 1539–1547.

Gold, K. 1976. Methods for preserving tintinnids. Pp. 236–239 in *Zooplankton Fixation and Preservation.* H. F. Steedman, ed. UNESCO, Paris.

Gould, S. J., and C. B. Calloway. 1980. Clams and brachiopods— ships that pass in the night. *Paleobiology* **6:** 383–396.

Hertlein, L. G., and U. S. Grant. 1944. The cenozoic Brachiopoda of western North America. *Publ. UCLA Math. Phys. Sci.* **3:** 1–236.

Jablonksi, D., and R. A. Lutz. 1983. Larval ecology of marine benthic invertebrates: paleobiological implications. *Biol. Rev.* **58:** 21–89.

James, M. A., A. D. Ansell, M. J. Collins, G. B. Curry, L. S. Peck, and M. C. Rhodes. 1992. Biology of living brachiopods. *Adv. Mar. Biol.* **28:** 175–387.

Law, R. H., and C. W. Thayer. 1991. Articulate fecundity in the phanerozoic: Steady state or what? Pp. 183–190 in *Brachiopods Through Time.* D.I. McKinnon, D. E. Lee, and J. D. Campbell, eds. Balkema, Rotterdam.

Long, J. A. 1964. The embryology of three species representing three superfamilies of articulate Brachiopoda. Ph.D. Dissertation, University of Washington, Seattle. 185 pp.

Long, J. A., and S. A. Stricker. 1991. Brachiopoda. Pp. 47–84 in *Reproduction in Marine Invertebrates, Vol. 6, Echinoderms and Lophophorates.* A. C. Giese, J. S. Pearse, and V. B. Pearse, eds. Boxwood Press, Palo Alto, CA.

Mano, R. 1960. On the metamorphosis of the brachiopod *Frenulina sanguinolenta* (Gmelin). *Bull. Mar. Biol. Stn. Asamushi* **10:** 171–175.

Mattox, N. T. 1955. Observations on the brachiopod communities near Santa Catalina Island. Pp. 73–86 in *Essays in the Natural Sciences in Honor of Captain Allan Hancock.* University of Southern California Press, Los Angeles.

Mileikovsky, S. A. 1973. Speed of active movement of pelagic larvae of marine bottom invertebrates and their ability to regulate their vertical position. *Mar. Biol.* **23:** 11–17.

Noble, J. P. A., A. Logan, and G. R. Webb. 1976. The recent *Terebratulina* community in the rocky subtidal zone of the Bay of Fundy, Canada. *Lethaia* **9:** 1–17.

Peck, L. S., and K. Robinson. 1994. Pelagic larval development in the brooding Antarctic brachiopod *Liothyrella uva. Mar. Biol.* **120:** 279–286.

Percival, E. 1944. A contribution to the life-history of the brachiopod *Terebratella inconspicua* Sowerby. *Trans. R. Soc. N. Z.* **74:** 1–23.

Percival, E. 1960. A contribution to the life-history of the brachiopod *Tegulorhynchia nigricans. Q. J. Microsc. Sci.* **101:** 439–458.

Reed, C. G. 1987. Phylum Brachiopoda. Pp. 486–493 in *Reproduction and Development of Marine Invertebrates of the Northern Pacific Coast,* M. F. Strathmann, ed. University of Washington Press, Seattle.

Rhodes, M. C., and C. W. Thayer. 1991. Effects of turbidity on suspension feeding: Are brachiopods better than bivalves? Pp. 191–196 in *Brachiopods Through Time,* D. I. McKinnon, D. E. Lee, and J. D. Campbell, eds. Balkema, Rotterdam.

Rhodes, M. C., and R. J. Thompson. 1993. Comparative physiology of suspension-feeding in living brachiopods and bivalves: evolutionary implications. *Paleobiology* **19:** 322–334.

Rudwick, M. J. S. 1970. *Living and Fossil Brachiopods.* Hutchinson, London. 199 pp.

Rumrill, S. S. 1990. Natural mortality of marine invertebrate larvae. *Ophelia* **32:** 163–198.

Stanley, S. M. 1977. Trends, rates and patterns of evolution in the Bivalvia. Pp 209–250 in *Patterns of Evolution.* A. Hallam, ed. Elsevier, Amsterdam.

Stricker, S. A., and M. W. Folsom. 1997. Oocyte maturation in the brachiopod *Terebratalia transversa:* role of follicle cell-oocyte attachments during ovulation and germinal vesicle breakdown. *Biol. Bull.* **193:** 324–340.

Stricker, S. A., and C. G. Reed. 1985a. The ontogeny of shell secretion in *Terebratalia transversa* (Brachiopoda, Articulata). I. Development of the mantle. *J. Morphol.* **183:** 233–250.

Stricker, S. A., and C. G. Reed. 1985b. The ontogeny of shell secretion in *Terebratalia transversa* (Brachiopoda, Articulata). II. Formation of the protegulum and juvenile shell. *J. Morphol.* **183:** 251–272.

Stricker, S. A., and C. G. Reed. 1985c. Development of the pedicle in the articulate brachiopod *Terebratalia transversa* (Brachiopoda, Terebratulida). *Zoomorphology* **105:** 253–264.

Thayer, C. W. 1981. Ecology of living brachiopods. Pp. 110–126 in *Lophophorates. Notes for a Short Course.* J. T. Dutro, Jr., and R. S. Boardman, eds. University of Tennessee, Knoxville. Publication EO1-1040-006-89.

Thayer, C. W. 1985. Brachiopods versus mussels: competition, predation, and palatability. *Science* **228:** 1527–1528.

Thayer, C. W. 1986. Are brachiopods better than bivalves? Mechanisms of turbidity tolerance and their interaction with feeding in articulates. *Paleobiology* **12:** 161–174.

Thayer, C. W., and R. A. Allmon. 1991. Unpalatable thecideid brachiopods from Palau: ecological and evolutionary implications. Pp. 253–260 in *Brachiopods Through Time.* D. I. McKinnon, D. E. Lee, and J. D. Campbell, eds. Balkema, Rotterdam.

Thorson, G. 1964. Light as an ecological factor in the dispersal and settlement of larvae of marine bottom invertebrates. *Ophelia:* **1:** 167–208.

Tunnicliffe, V., and K. Wilson. 1988. Brachiopod populations: distribution in fjords of British Columbia (Canada) and tolerance of low oxygen concentrations. *Mar. Ecol. Prog. Ser.* **47:** 117–128.

Valentine, J. W., and D. Jablonski. 1983a. Larval adaptations and patterns of brachiopod diversity through time. *Evolution* **37:** 1052–1061.

Valentine, J. W., and D. Jablonski. 1983b. Speciation in the shallow sea: general patterns and biogeographic controls. Pp. 201–226 in *Evolution, Time and Space: The Emergence of the Biosphere.* R. W. Sims, J. H. Price, and P. E. S. Whalley, eds. Academic Press, New York.

Vermeij, G. J. 1977. The Mesozoic marine revolution: evidence from snails, predators and grazers. *Paleobiology* **3:** 245–258.

Wisely, B. 1969. Preferential settlement in concavities by the brachiopod *Waltonia inconspicua. N. Z. J. Mar. Freshwater Res.* **3:** 273–280.

Young, C. M., and F. S. Chia. 1987. Abundance and distribution of pelagic larvae as influenced by predation, behavior, and hydrographic factors. Pp. 385–464 in *Reproduction of Marine Invertebrates, Vol. IX.* A. C. Giese, J. S. Pearse, and V. B. Pearse, eds. Blackwell Scientific, Palo Alto, CA.

Zimmer, R. L., and E. C. Haderlie. 1980. Brachiopoda and phoronida. Pp. 108–114 in *Intertidal Invertebrates of California.* R. H. Morris, D. P. Abbott, and E. C. Haderlie, eds. Stanford University Press, Stanford, CA.